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Award Number: W81XWH-11-1-0809

TITLE: Potential Environmental Triggers of Myositis

PRINCIPAL INVESTIGATOR: Robert Goldberg

CONTRACTING ORGANIZATION: The Mytosis Association, Alexandria, VA 22314

REPORT DATE: October 2012

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

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				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
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14. ABSTRACT Children's Research Institute has been able to complete whole genome methylation analysis on the available biopsies and has identified a short list of genes which are significantly different between healthy patients at the epigenetic level.					
15. SUBJECT TERMS- genes, methylation analysis					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 25	19a. NAME OF RESPONSIBLE PERSON USAMRMC
a. REPORT U	b. ABSTRACT U	c. THIS PAGE U			19b. TELEPHONE NUMBER (include area code)

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INTRODUCTION:

The Potential Environmental Triggers of Myositis project consists of two projects:

1. Environment induced epigenetic changes in skeletal muscle and their role in myositis pathogenesis
2. Environmental Risk Factors for the Development of Myositis in Military Personnel

The following section, "Body," contains the approved Aims of the project as contained in the statement of work for project #1 listed above. Project #2 had its start delayed by 1 year and the revised application and budget was approved with a new start date of October 1, 2012. The approval of modification P00002 was received on October 26, 2012 (see Appendix 1). Thus, there is nothing to report for project #2 in this Annual Report.

BODY

Environment induced epigenetic changes in skeletal muscle and their role in myositis pathogenesis

Progress Report for Year 1

Purpose: With our Aim 1 we proposed to characterize the epigenetic changes and resulting gene expression disturbances in myositis patient samples at the whole genome level. With our collaborators at the Karolinska Institute and Genpathway, Inc. we will perform genome-wide and gene expression and methylation analysis to develop a computational data integration system. This combination of gene expression and epigenetic data (DNA methylation) will identify altered gene expression due to methylation. Using this technology we will perform and analyze global DNA methylation changes in well characterized myositis (6 PM and 6 DM) and control (6 normal) muscle biopsies. With Aim 2 we proposed to examine whether environmental agents (e.g. statin drugs or viral infections) could induce epigenetic changes *in vitro*. For this work, we will make use of human primary muscle cells and test their response to two statin drugs as well as infection by Cocksackie B6 virus. This work will be performed in house at CNMC.

We hypothesized that exposure to environmental agents could result in DNA methylation changes in the genome leading to aberrant expression of certain genes (e.g. MHC class I) that initiate and sustain muscle inflammation in individuals with susceptible genetic loci.

At the present time we have been able to complete whole genome methylation analysis on our available biopsies and have identified a short list of genes which are significantly different between healthy patients and myositis patients at the epigenetic level.

Aim 1 Samples: For this analysis we were given biopsies from two different groups of patients. The first group consisted of healthy female controls, and the second group consisted of patient with polymyositis and dermatomyositis. Healthy controls were composed of 5 women (mean age 58.2 ± 2.8 years) while myositis patients consisted of 4 male and 6 female patients (mean age 62.5 ± 1.9 years). A

Table 1: Patient Biopsy Demographics

Patient #	Gender	Age	Diagnosis	Rxn Response
8848D	Female	54	Control	N/A
8847D	Female	62	Control	N/A
8846D	Female	56	Control	N/A
8845D	Female	67	Control	N/A
8843D	Female	52	Control	N/A
200	Female	61	DM	Non-responder
144	Female	55	DM	Non-responder
113	Female	60	DM	Non-responder
446	Female	67	DM	Responder
71	Male	65	DM	Responder
722	Male	60	PM	Responder
264	Female	71	PM	Responder
100	Male	71	PM	Responder
93	Male	62	PM	Responder
12	Female	53	PM	Non-responder

breakdown of the individual patient samples is provided below, where DM = dermatomyositis and PM = polymyositis.

All patients diagnosed with either PM or DM were treated with prednisone, while some received methotrexate in addition. Patient biopsies were obtained by the Karolinska Institute in Stockholm, Sweden.

Aim 1 Methods: A total of 500 ng of genomic DNA was used for bisulfate conversion using a Bisulfite Conversion Kit (Invitrogen) according to the manufacturer's protocol. Bisulfite conversion converts

unmethylated cytosine bases into uracil bases, while methylated cytosines escape conversion. Following bisulfite conversion, the genomic DNA was hybridized onto an Illumina Human 450K Methylation BeadChip. This array is capable of examining roughly 450,000 individual methylation loci per patient. Data was collected using the GenomeStudio software (Illumina) and exported to Partek for third party statistical analysis. For statistical analysis, a methylation threshold of (beta > 0.1) was used and all loci on the X chromosome were ignored to remove gender-specific bias. Further analysis carried out by our collaborator Dr. Joseph Devaney allowed us to collate (or "tile") these individual loci into groups centered on CpG islands in the genome.

Aim 1 Results: To date we have been able to obtain biopsies for 15 of our planned 18 samples and have successfully performed genome-wide detection of differential methylation states.

Table 2: Methylation changes in Healthy vs. Disease

Healthy Methylated %	Myositis Methylated %	Gene
18.41	63.80	PXDN
23.47	77.42	PHACTR4
36.88	59.42	KIF26A
39.35	61.61	CD36
39.06	60.27	DSCAM
38.73	60.76	ABL1
37.79	60.61	KNDC1
36.32	59.11	PLIN1
61.79	38.11	EN2
66.76	33.11	SIM2
70.97	23.77	SLC37A1
65.61	29.09	KCTD5

Table 3: Methylation in Responders vs. Non-responders

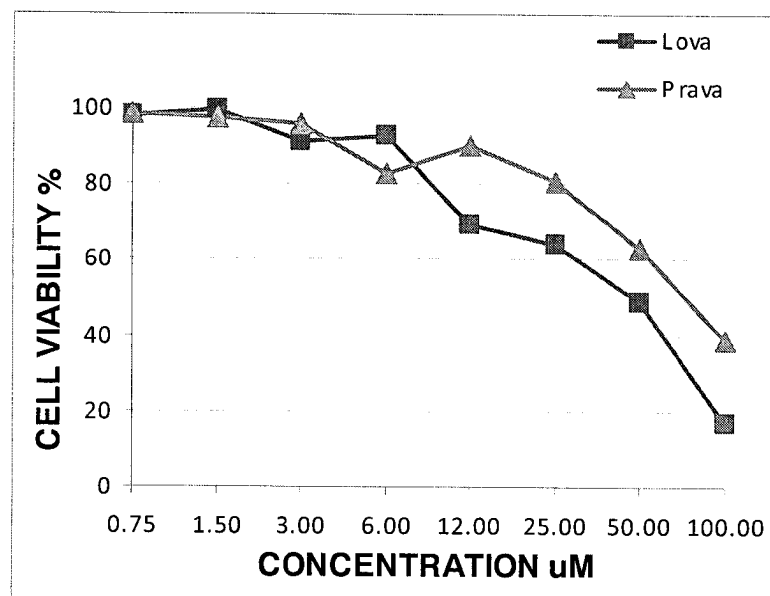
Responder Methylated %	Non-responder Methylated %	Gene
61.84	39.02	SORCS2
60.25	37.98	TRIM8
66.60	37.61	CCAR1
70.03	38.68	TNXB
66.10	36.37	CD52
64.00	13.71	SERPINA9

in Tables 2 and 3 have not previously been implicated in myositis, prior literature reports the genes PHACTR4, SIM2, SLC37A1, SORCS2, CCAR1, and TNXB all belong to pathways known to be important for normal muscle functions or associated with the pathogenesis of myositis.

Aim2 Methods: Human primary skeletal muscle cells (SkMCs) were purchased from Lonza (Clonetics SkMC Human Skeletal Muscle Cells). Live stocks of the Coxsackie B6 virus were obtained from ATCC. The compounds lovastatin (M2147) and pravastatin (P4498) were

Preliminary analysis using Partek indicated that a large number of methylation loci were not different between groups. Nevertheless, a total of 12,750 CpG loci were found to be significantly different between healthy controls and all myositis patients. Furthermore, an additional 10,683 loci were found to be differentially methylated between responders and non-responders. After these loci were tiled, we were able to identify 12 individual genes that were significantly different between healthy and disease groups, while 6 genes were found to be significantly different between responder and non-responder groups. These results are encouraging considering the small sample size available. Obtaining additional biopsies should allow us to both identify additional genes of interest and also validate these genes as potential therapeutic targets. While each of the genes listed

purchased from Sigma-Aldrich. The cells were cultured in growth medium (GIBCO Ham's F-10 nutrient mix, 20% fetal bovine serum, 2% chicken embryo extract and 1% penicillin/streptomycin) at 37°C in a humidified chamber with 5% CO₂. Next the muscle cells were induced to differentiate into myotubes (5 days differentiation) by switching to differentiation medium [DMEM (4500 mg/l glucose, 4mM L-glutamine, 110 mg/l pyruvate), 5% horse serum, 2% L-glutamine, 1% penicillin/streptomycin]. For experiments, additional stocks of virus were obtained by infecting cultures of either HeLa cells or SkMCs and collecting culture supernatant. A viral titer of 63.0×10^6 pfu was obtained from infected HeLa cells, while a viral titer of 1.9×10^6 pfu was obtained from infected SkMCs.



Aim 2 Results: Experiments were conducted to determine dose response curves for lovastatin and pravastatin using cell viability (MTT assay) assays. These experiments allowed us to determine the maximal drug concentration that can be used *in vitro* to test possible induced epigenetic changes in human skeletal muscle cells (SkMCs). The concentration range tested for both lovastatin and pravastatin was 0.75-100 μM lovastatin. Initial attempts to identify an optimal drug dosage were slowed by problems of drug solubility and reactivity in solution. These issues have been

solved, and the optimal drug concentrations for both lovastatin and pravastatin were determined to be between 3 and 6 μM. For future experiments we will use 6 μM of drug in culture to examine possible effects on DNA methylation.

Future plans: In the immediate future we will perform pyrosequencing to validate the methylation data for our genes of interest described in the results for Aim 1. Additionally, once we have completed whole genome gene expression analysis, these studies will allow us to identify pathways in myositis patients that are known to be affected by epigenetic changes. We will validate these findings both at mRNA and protein level in additional patient muscle biopsies. These findings will help to define role and possible pathologic mechanisms for environmental agents in myositis disease onset and perpetuation. For Aim 2 statins experiments, we are carrying out the proposed *in vitro* treatment of SkMCs with both lovastatin and pravastatin. For Aim 2 viral experiments, we are currently performing *in vitro* experiments with SkMCs to determine the TCID₅₀ value for infection with Coxsackie B6 viral particles. After establishing the optimal dosage of viral particles, we will move on to the proposed treatment of SkMCs with Coxsackie B6. Following the completion of these treatments, we will analyze the genomic DNA from treated and untreated SkMCs to detect changes in epigenetic regulation.

KEY RESEARCH ACCOMPLISHMENTS

- Aim 1 was to characterize the epigenetic changes and resulting gene expression disturbances in myositis patient samples at the whole genome level. Following whole genome methylation analysis, it was found that 12 individual genes were significantly different between healthy and disease groups, while 6 genes were found to be significantly different between responder and non-responder groups. These results are encouraging considering the small sample size available.
- Aim 2 was to examine whether environmental agents (e.g. statin drugs or viral infections) could induce epigenetic changes *in vitro*. Experiments were conducted to determine dose response curves for lovastatin and pravastatin using cell viability (MTT assay) assays. These experiments determined the maximal drug concentration that can be used in vitro to test possible induced epigenetic changes in human skeletal muscle cells (SkMCs). The concentration range tested for both lovastatin and pravastatin and the optimal drug concentrations for both lovastatin and pravastatin were determined to be between 3 and 6 μM . For future experiments we will use 6 μM of drug in culture to examine possible effects on DNA methylation.

REPORTABLE OUTCOMES:

There are no reportable outcomes at this time.


CONCLUSION:

Satisfactory progress has been made toward Aims 1 & 2 in Project # 1: "Environment induced epigenetic changes in skeletal muscle and their role in myositis pathogenesis."

Pyrosequencing will be used to validate the methylation data for genes of interest and following whole genome gene expression analysis, these studies will allow the research to identify pathways in myositis patients that are known to be affected by epigenetic changes. These findings will be validated at the mRNA and protein level in additional patient muscle biopsies.

REFERENCES:

None.

AMENDMENT OF SOLICITATION/MODIFICATION OF CONTRACT				1. CONTRACT ID CODE S		PAGE OF PAGES 1 13	
2. AMENDMENT/MODIFICATION NO. P00002		3. EFFECTIVE DATE 26-Oct-2012		4. REQUISITION/PURCHASE REQ. NO. W23RYX1144N612		5. PROJECT NO. (If applicable)	
6. ISSUED BY US ARMY MEDICAL RESEARCH ACQUISITION ACT DIRECTOR 820 CHANDLER STREET FORT DETRICK MD 21702-5014		CODE W81XWH		7. ADMINISTERED BY (If other than item 6) US ARMY MEDICAL RESEARCH ACQUISITION ACT ATTN: MARIBEL FRATINA MARIBEL.FRATINA@US.ARMY.MIL FORT DETRICK MD 21702		CODE W81XWH	
8. NAME AND ADDRESS OF CONTRACTOR (No., Street, County, State and Zip Code) MYOSITIS ASSOCIATION OF AMERICA 1233 20TH ST NW LOWR WASHINGTON DC 20036-2346				9A. AMENDMENT OF SOLICITATION NO.			
				9B. DATED (SEE ITEM 11)			
				X 10A. MOD. OF CONTRACT/ORDER NO. W81XWH-11-1-0809			
				10B. DATED (SEE ITEM 13) X 12-Sep-2011			
CODE 52PC6		FACILITY CODE					
11. THIS ITEM ONLY APPLIES TO AMENDMENTS OF SOLICITATIONS							
<input type="checkbox"/> The above numbered solicitation is amended as set forth in Item 14. The hour and date specified for receipt of Offer <input type="checkbox"/> is extended, <input type="checkbox"/> is not extended. Offer must acknowledge receipt of this amendment prior to the hour and date specified in the solicitation or as amended by one of the following methods: (a) By completing Items 8 and 15, and returning _____ copies of the amendment; (b) By acknowledging receipt of this amendment on each copy of the offer submitted; or (c) By separate letter or telegram which includes a reference to the solicitation and amendment numbers. FAILURE OF YOUR ACKNOWLEDGMENT TO BE RECEIVED AT THE PLACE DESIGNATED FOR THE RECEIPT OF OFFERS PRIOR TO THE HOUR AND DATE SPECIFIED MAY RESULT IN REJECTION OF YOUR OFFER. If by virtue of this amendment you desire to change an offer already submitted, such change may be made by telegram or letter, provided each telegram or letter makes reference to the solicitation and this amendment, and is received prior to the opening hour and date specified.							
12. ACCOUNTING AND APPROPRIATION DATA (If required)							
13. THIS ITEM APPLIES ONLY TO MODIFICATIONS OF CONTRACTS/ORDERS. IT MODIFIES THE CONTRACT/ORDER NO. AS DESCRIBED IN ITEM 14.							
A. THIS CHANGE ORDER IS ISSUED PURSUANT TO: (Specify authority) THE CHANGES SET FORTH IN ITEM 14 ARE MADE IN THE CONTRACT ORDER NO. IN ITEM 10A.							
B. THE ABOVE NUMBERED CONTRACT/ORDER IS MODIFIED TO REFLECT THE ADMINISTRATIVE CHANGES (such as changes in paying office, appropriation date, etc.) SET FORTH IN ITEM 14, PURSUANT TO THE AUTHORITY OF FAR 43.103(B).							
C. THIS SUPPLEMENTAL AGREEMENT IS ENTERED INTO PURSUANT TO AUTHORITY OF:							
X D. OTHER (Specify type of modification and authority) Mutual agreement.							
E. IMPORTANT: Contractor <input checked="" type="checkbox"/> is not, <input type="checkbox"/> is required to sign this document and return _____ copies to the issuing office.							
14. DESCRIPTION OF AMENDMENT/MODIFICATION (Organized by UCF section headings, including solicitation/contract subject matter where feasible.) Modification Control Number: mfratina127239 1. The purpose of subject modification is to incorporate the recipient's revised budget and advance payment schedule; in accordance with their request, which is incorporated herein by reference. 2. The Grants Officer's Representative, Dr. John Carney has approved the above via email dated 22 October 2012. 3. Subject modification also incorporates award close-out procedures. 4. This modification is at no additional cost to the government. 5. See Summary of Changes for details. 6. All other terms and conditions remain unchanged.							
Except as provided herein, all terms and conditions of the document referenced in Item 9A or 10A, as heretofore changed, remains unchanged and in full force and effect.							
15A. NAME AND TITLE OF SIGNER (Type or print)				16A. NAME AND TITLE OF CONTRACTING OFFICER (Type or print) PAMELA NEVELS / ACCOUNT MANAGER TEL: 301-619-8802 EMAIL: pamelanevels@us.army.mil			
15B. CONTRACTOR/OFFEROR (Signature of person authorized to sign)		15C. DATE SIGNED		16B. UNITED STATES OF AMERICA BY  (Signature of Contracting Officer)		16C. DATE SIGNED 26-Oct-2012	

SECTION SF 30 BLOCK 14 CONTINUATION PAGE

SUMMARY OF CHANGES

SECTION 00010 - SOLICITATION CONTRACT FORM

The following have been modified:

PI NAME & PROPOSAL TITLE

Principal Investigator: Mr. Robert Goldberg

Proposal Title: "Potential Environmental Triggers of Myositis"

SECTION 00800 - SPECIAL CONTRACT REQUIREMENTS

The following have been modified:

A. This award is made under the authority of 31 U.S.C. 6304 and 10 U.S.C. 2358. The recipient's statement of work and the revised budget submitted 25 May 2011 of the proposal dated 27 April 2011 are incorporated herein by reference. The Catalog of Federal Domestic Assistance Number relative to this award is CFDA 12.420.

Modification P00002 incorporates the revised budget submitted 1 October 2012.

B. ACCEPTANCE OF AWARD: The recipient is not required to countersign this assistance award. In case of disagreement, the recipient shall notify the Grants Officer and not assess the award any costs until such disagreement(s) is resolved.

C. USAMRAA GENERAL TERMS AND CONDITIONS: This assistance agreement is subject to the USAMRAA General Terms and Conditions and to any special considerations as contained in the below mentioned Section titled "Special Terms and Conditions". These USAMRAA General Terms and Conditions are incorporated by reference with the same force and effect as if they were given in full text. The full text of the USAMRAA General Terms and Conditions may be accessed electronically at <http://www.usamraa.army.mil>.

D. SPECIAL TERMS AND CONDITIONS

1. TECHNICAL REPORTING REQUIREMENTS (DEC 2008) (USAMRAA)

PROGRAMMATIC LINE REVIEW (PLR)

a. The reporting requirements for Telemedicine and Advanced Technology Research Center (TATRC) include quarterly, annual and final reports and the Principal Investigator's (PI's) participation in at least one programmatic line review (PLR) for this project each year of the project's period-of-performance.

b. The PI shall prepare for and participate in at least one PLR for this project for each year of the project's term, at the Grants Officer's Representative's (GOR's) request. The invitation and format for the programmatic

review will be provided by TATRC at least 90 days prior to the meeting. The meetings will generally be held in the Fort Detrick, Maryland, area, but may occur elsewhere in the U.S. Participation in the PLR will be in lieu of submitting next scheduled Quarterly report required under the award.

QUARTERLY REPORTS

a. Quarterly reports are the most immediate and direct contact between the Principal Investigator (PI) and the Grants Officer's Representative (GOR). The reports provide the means for keeping this Command advised of developments and problems as the research effort proceeds. The quarterly reports also provide a measure against which decisions on release of funding and on requests for supplements are made.

b. In accordance with Section C., a Quarterly Report shall be submitted for each three-month period beginning with the effective date of the assistance agreement. This requirement includes all three-month periods of the assistance agreement.

c. Copies of each report shall be submitted in the quantities indicated to the addresses shown below within fifteen (15) days after the end of each quarter. Internal Government distribution will be made by those offices (electronic submission preferred).

(1) One (1) copy of the report to:

Grants Officer's Representative: Dr. John Carney
Telemedicine and Advanced Technology Research Center (TATRC)
504 Scott Street
Fort Detrick, Maryland 21702
Email: john.carney@tatrc.org

(2) One (1) copy of the report to:

Grants Officer's Representative
U.S. Army Medical Research Acquisition Activity
ATTN: MCMR-AAA-T (W81XWH-11-1-0809)
820 Chandler Street
Fort Detrick, MD 21702-5014
Email: maribel.fratina@amedd.army.mil

(3) One (1) copy of the report to:

USAMRAA.TAN@amedd.army.mil

d. The Quarterly Report sample (See following Quarterly Report Format) shall serve as the format. Each item of the report format shall be completed.

QUARTERLY REPORT FORMAT

1. Award No. _____ 2. Report Date _____

3. Reporting period from _____ to _____

4. PI _____ 5. Telephone No. _____

6. Institution _____

7. Project Title _____

8. Current staff, with percent effort of each on project.

_____% _____%

_____% _____%

9. Award expenditures to date (as applicable):

This Qtr/Cumulative**This Qtr/Cumulative**

Personnel _____/_____ Travel _____/_____

Fringe Benefits _____/_____ Equipment _____/_____

Supplies _____/_____ Other _____/_____

This Qtr/Cumulative

Subtotal _____/_____

Indirect Costs _____/_____

Fee _____/_____

Total _____/_____

10. Comments on administrative and logistical matters.

11. Use additional page(s), as necessary, to describe scientific progress for the quarter in terms of the tasks or objectives listed in the statement of work for this assistance agreement.

12. Use additional page(s) to present a brief statement of plans or milestones for the next quarter.

TECHNICAL REPORTING REQUIREMENTS (DEC 2008) (USAMRAA)

Format Requirements for Annual/Final Reports

a. Annual reports must provide a complete summary of the research accomplishments to date with respect to the approved Statement of Work. Journal articles can be substituted for detailed descriptions of specific aspects of the research, but the original articles must be attached to the report as an appendix and appropriately referenced in the text. The importance of the report to decisions relating to continued support of the research can not be over-emphasized. An annual report shall be submitted within 30 calendar days of the anniversary date of the award for the preceding 12 month period. If the award period of performance is extended by the Grants Officer, then an annual report must still be submitted within 30 days of the anniversary date of the award. A final report will be due upon completion of the extended performance date that describes the entire research effort.

b. A final report summarizing the entire research effort, citing data in the annual reports and appended publications shall be submitted at the end of the award performance period. The final report will provide a complete reporting of the research findings. Journal publications can be substituted for detailed descriptions of specific aspects of the research, but an original copy of each publication must be attached as an appendix and appropriately referenced in the text. All final reports must include a bibliography of all publications and meeting abstracts and a list of personnel (not salaries) receiving pay from the research effort.

Although there is no page limitation for the reports, each report shall be of sufficient length to provide a thorough description of the accomplishments with respect to the approved Statement of Work. Submission of the report in electronic format (PDF or Word file only), shall be submitted to <https://ers.amedd.army.mil>.

All reports shall have the following elements in this order

FRONT COVER: Sample front cover provided at <https://mrmc.amedd.army.mil/rpindex.asp>. The Accession Document (AD) Number should remain blank.

STANDARD FORM 298: Sample SF 298 provided at <https://mrmc.amedd.army.mil/rpindex.asp>. The abstract in Block 13 must state the purpose, scope, major findings and be an up-to-date report of the progress in terms of results and significance. Subject terms are keywords that may have previously assigned to the proposal abstract or are keywords that may be significant to the research. The number of pages shall include all pages that have printed data (including the front cover, SF 298, table of contents, and all appendices). Please count pages carefully to ensure legibility and that there are no missing pages as this delays processing of reports. Page numbers should be typed: please do not hand number pages.

TABLE OF CONTENTS: Sample table of contents provided at <https://mrmc.amedd.army.mil/rpindex.asp>.

INTRODUCTION: Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.

BODY: This section of the report shall describe the research accomplishments associated with each task outlined in the approved Statement of Work. Data presentation shall be comprehensive in providing a complete record of the research findings for the period of the report. Provide data explaining the relationship of the most recent findings with that of previously reported findings. Appended publications and/or presentations may be substituted for detailed descriptions of methodology but must be referenced in the body of the report. If applicable, for each task outlined in the Statement of Work, reference appended publications and/or presentations for details of result findings and tables and/or figures. The report shall include negative as well as positive findings. Include problems in accomplishing any of the tasks. Statistical tests of significance shall be applied to all data whenever possible. Figures and graphs referenced in the text may be embedded in the text or appended. Figures and graphs can also be referenced in the text and appended to a publication. Recommended changes or future work to better address the research topic may also be included, although changes to the original Statement of Work must be approved by the

Army Grants Officer's Representative. This approval must be obtained prior to initiating any change to the original Statement of Work.

KEY RESEARCH ACCOMPLISHMENTS: Bulleted list of key research accomplishments emanating from this research.

REPORTABLE OUTCOMES: Provide a list of reportable outcomes that have resulted from this research to include:

manuscripts, abstracts, presentations; patents and licenses applied for and/or issued; degrees obtained that are supported by this award; development of cell lines, tissue or serum repositories; informatics such as databases and animal models, etc.; funding applied for based on work supported by this award; employment or research opportunities applied for and/or received based on experience/training supported by this award.

CONCLUSION: Summarize the results to include the importance and/or implications of the completed research and when necessary, recommend changes on future work to better address the problem. A "so what section" which evaluates the knowledge as a scientific or medical product shall also be included in the conclusion of the report.

REFERENCES: List all references pertinent to the report using a standard journal format (i.e. format used in *Science, Military Medicine*, etc.).

APPENDICES: Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

Pages shall be consecutively numbered throughout the report. **DO NOT RENUMBER PAGES IN THE APPENDICES.**

Mark all pages of the report which contain proprietary or unpublished data that should be protected by the U.S. Government. **REPORTS NOT PROPERLY MARKED FOR LIMITATION WILL BE DISTRIBUTED AS APPROVED FOR PUBLIC RELEASE.** It is the responsibility of the Principal Investigator to advise the U.S. Army Medical Research and Materiel Command when restricted limitation assigned to a document can be downgraded to Approved for Public Release. **DO NOT USE THE WORD "CONFIDENTIAL" WHEN MARKING DOCUMENTS.**

2. ADVANCE PAYMENTS AND FULL FUNDING (APRIL 2011) (USAMRAA)

a. Payments. Advance payments will be made to the recipient. Questions relative to payment issues involving Defense Finance and Accounting Service shall be directed to USAMRAA.TAN@AMEDD.ARMY.MIL.

b. Electronic Funds Transfer. All advance payments to the recipient will be made by electronic funds transfer (EFT) to the recipient's financial institution account listed in the Central Contractor Registry (CCR). Failure to update CCR may result in nonpayment.

c. If the recipient fails to perform, the Grants Officer shall notify DFAS in writing to withhold payments.

d. Advance Payment Schedule

Year One \$174,799.84

<u>Amount</u>	<u>On or About</u>
\$43,699.96	Upon execution of this award (PAID)
\$43,699.96	12 December 2011 (PAID)

\$43,699.96	12 March 2012 (PAID)
\$43,699.96	12 June 2012 (PAID)

Year Two \$179,034.84

<u>Amount</u>	<u>On or About</u>
\$43,699.96	12 September 2012 (PAID)
\$45,817.46	12 December 2012
\$44,758.71	12 March 2013
\$44,758.71	12 June 2013

Year Three \$179,384.86

<u>Amount</u>	<u>On or About</u>
\$44,846.22	12 September 2013
\$44,846.22	12 December 2013
\$44,846.22	12 March 2014
\$44,846.20	12 June 2014

Year Four \$177,792.30

<u>Amount</u>	<u>On or About</u>
\$44,448.08	12 September 2014
\$44,448.08	12 December 2014
\$44,448.07	12 March 2015
\$44,448.07	12 June 2015

Year Five \$162,987.30

<u>Amount</u>	<u>On or About</u>
\$40,746.83	12 September 2015
\$40,746.83	12 December 2015
\$40,746.82	12 March 2016
\$40,746.82	12 June 2016

e. Financial Reporting Requirements:

Federal Financial Report (SF 425): Quarterly and Final Reports (For reporting individual assistance agreements)

Reporting period end dates fall on the end of the calendar quarter for quarterly reports (3/31, 6/30, 9/30, 12/31) and the end date of the assistance agreement period of performance for the final report. Reports are due 30 days after the reporting period end date for quarterly reports and 90 days after the end date of the assistance agreement for the final report.

The SF425 and instructions for completion can be obtained from <https://usamraa.army.mil>. All SF425's shall be submitted electronically to USAMRAASF425@amedd.army.mil. The award number assigned by USAMRAA, which looks similar to W81XWH-XX-X-XXXX shall be included in the subject line of the electronic submission.

NOTE: The SF425 is a single form that consolidates and replaces the Federal Cash Transaction Report (SF272.SF272A) and the Financial Status Report (SF269/SF269A)

f. Interest Bearing Account. Unless exempted by applicable Treasury-State agreements in accordance with the Cash Management Improvement Act (CMIA) (31 U.S.C. 3335), the recipient shall deposit all advance payments in an interest bearing account. Interest over the amount of \$250 per year shall be remitted annually to the Department of Health and Human Services, Payment Management System, P.O. Box 6021, Rockville, MD 20852. A copy of the transmittal letter stating the amount of interest remitted shall be sent to the U.S. Army Medical Research Acquisition Activity, ATTN:MCMR-AAA-T, 820 Chandler Street, Fort Detrick, MD 21702-5014.

3. PROHIBITION OF USE OF LABORATORY ANIMALS (JAN 2007) (USAMRAA)

**** PROHIBITION – READ FURTHER FOR DETAILS ****

Notwithstanding any other provisions contained in this award or incorporated by reference herein, the contractor is expressly forbidden to use or subcontract for the use of laboratory animals in any manner whatsoever without the express written approval of the US Army Medical Research and Materiel Command, Animal Care and Use Office (ACURO). The contractor will receive written approval to begin research under the applicable protocol proposed for this award from the US Army Medical Research and Materiel Command, ACURO, under separate letter. A copy of this approval will be provided to the US Army Medical Research and Acquisition Activity for the official file. Non-compliance with any provision of this clause may result in the termination of the award.

4. PROHIBITION OF HUMAN RESEARCH (JAN 2007) (USAMRAA)

**** PROHIBITION – READ FURTHER FOR DETAILS ****

Research under this award involving the use of human subjects, to include the use of human anatomical substances and/or human data, may not begin until the U.S. Army Medical Research and Materiel Command's Office of Research Protections, Human Research Protections Office (HRPO) approves the protocol. Written approval to begin research or subcontract for the use of human subjects under the applicable protocol proposed for this award will be issued from the US Army Medical Research and Materiel Command, HRPO, under separate letter to the contractor. A copy of this approval will be provided to the US Army Medical Research Acquisition Activity for the official file. Non-compliance with any provision of this clause may result in withholding of funds and or the termination of the award.

5. PROHIBITION OF USE OF HUMAN CADAVERS (JAN 2007) (USAMRAA)

**** PROHIBITION – READ FURTHER FOR DETAILS****

Research under this award using human cadavers may not begin until the U.S. Army Medical Research and Materiel Command's Office of Research Protections, Human Research Protections Office (HRPO) approves the protocol. Written approval to begin research or subcontract for the use of human cadavers under the applicable protocol proposed for this award will be issued from the US Army Medical Research and Materiel Command, HRPO, under separate letter to the contractor. A copy of this approval will be provided to the US Army Medical Research Acquisition Activity for the official file. Non-compliance with any provision of this clause may result in withholding of funds and or the termination of the award.

6. MAXIMUM OBLIGATION (SEP 2006) (USAMRAA)

The maximum obligation for support of the project will not exceed the amount specified in the award, as amended. USAMRAA does not amend assistance agreements to provide additional funds for such purposes as reimbursement for unrecovered indirect costs resulting from the establishment of final negotiated rates or for increases in salaries, fringe benefits and other costs.

7. SUPPORTING INFORMATION (APR 2008) (USAMRAA)

Information such as subawards, consultant agreements, vendor quotes, and personnel work agreements may be required in order to support proposed costs or to determine the employment status of personnel under the assistance agreement. The Government's receipt of this information does not constitute approval or acceptance of any term or condition included therein. The terms and conditions of the assistance agreement take precedence over any term or condition included in supporting information.

8. REQUIREMENTS FOR FEDERAL FUNDING ACCOUNTABILITY AND TRANSPARENCY ACT IMPLEMENTATION (2 CFR Part 170)

Appendix A to Part 170--Award Term

I. Reporting Subawards and Executive Compensation

A. Reporting of first-tier subawards.

1. Applicability. Unless you are exempt as provided in paragraph D. of this award term, you must report each action that obligates \$25,000 or more in Federal funds that does not include Recovery funds (as defined in section 1512(a)(2) of the American Recovery and Reinvestment Act of 2009, Pub. L. 111-5) for a subaward to an entity (see definitions in paragraph e. of this award term).

2. Where and when to report.

i. You must report each obligating action described in paragraph a.1. of this award term to <http://www.fsrc.gov>.

ii. For subaward information, report no later than the end of the month following the month in which the obligation was made. (For example, if the obligation was made on November 7, 2010, the obligation must be reported by no later than December 31, 2010.)

3. What to report. You must report the information about each obligating action that the submission instructions posted at <http://www.fsrc.gov> specify. must report the information about each obligating action that the submission instructions posted at <http://www.fsrc.gov> specify.

B. Reporting Total Compensation of Recipient Executives.

1. Applicability and what to report. You must report total compensation for each of your five most highly compensated executives for the preceding completed fiscal year, if--

- i. the total Federal funding authorized to date under this award is \$25,000 or more;
- ii. in the preceding fiscal year, you received—

(A) 80 percent or more of your annual gross revenues from Federal procurement contracts (and subcontracts) and Federal financial assistance subject to the Transparency Act, as defined at 2 CFR 170.320 (and subawards); and

(B) \$25,000,000 or more in annual gross revenues from Federal procurement contracts (and subcontracts) and Federal financial assistance subject to the Transparency Act, as defined at 2 CFR 170.320 (and subawards); and

iii. The public does not have access to information about the compensation of the executives through periodic reports filed under section 13(a) or 15(d) of the Securities Exchange Act of 1934 (15 U.S.C. 78m(a), 78o(d)) or section 6104 of the Internal Revenue Code of 1986. (To determine if the public has access to the compensation information, see the U.S. Security and Exchange Commission total compensation filings at <http://www.sec.gov/answers/execomp.htm>.)

2. Where and when to report. You must report executive total compensation described in paragraph b.1. of this award term:

- i. As part of your registration profile at <http://www.ccr.gov>.
- ii. By the end of the month following the month in which this award is made, and

annually thereafter.

C. Reporting of Total Compensation of Subrecipient Executives.

1. Applicability and what to report. Unless you are exempt as provided in paragraph d. of this award term, for each first-tier subrecipient under this award, you shall report the names and total compensation of each of the subrecipient's five most highly compensated executives for the subrecipient's preceding completed fiscal year, if--

i. in the subrecipient's preceding fiscal year, the subrecipient received--

(A) 80 percent or more of its annual gross revenues from Federal procurement contracts (and subcontracts) and Federal financial assistance subject to the Transparency Act, as defined at 2 CFR 170.320 (and subawards); and

(B) \$25,000,000 or more in annual gross revenues from Federal procurement contracts (and subcontracts), and Federal financial assistance subject to the Transparency Act (and subawards); and

ii. The public does not have access to information about the compensation of the executives through periodic reports filed under section 13(a) or 15(d) of the Securities Exchange Act of 1934 (15 U.S.C. 78m(a), 78o(d)) or section 6104 of the Internal Revenue Code of 1986. (To determine if the public has access to the compensation information, see the U.S. Security and Exchange Commission total compensation filings at <http://www.sec.gov/answers/execomp.htm>.)

2. Where and when to report. You must report subrecipient executive total compensation described in paragraph c.1. of this award term:

i. To the recipient.

ii. By the end of the month following the month during which you make the subaward. For example, if a subaward is obligated on any date during the month of October of a given year (i.e., between October 1 and 31), you must report any required compensation information of the subrecipient by November 30 of that year.

D. Exemptions. If, in the previous tax year, you had gross income, from all sources, under \$300,000, you are exempt from the requirements to report:

i. Subawards, and

ii. The total compensation of the five most highly compensated executives of any subrecipient.

E. Definitions. For purposes of this award term:

1. Entity means all of the following, as defined in 2 CFR part 25:

i. A Governmental organization, which is a State, local government, or Indian tribe;

ii. A foreign public entity;

iii. A domestic or foreign nonprofit organization;

iv. A domestic or foreign for-profit organization;

v. A Federal agency, but only as a subrecipient under an award or subaward to a non-Federal entity.

2. Executive means officers, managing partners, or any other employees in management positions.

3. Subaward:

i. This term means a legal instrument to provide support for the performance of any portion of the substantive project or program for which you received this award and that you as the recipient

award to an eligible subrecipient.

ii. The term does not include your procurement of property and services needed to carry out the project or program (for further explanation, see Sec. ---- .210 of the attachment to OMB Circular A-133, "Audits of States, Local Governments, and Non-Profit Organizations").

iii. A subaward may be provided through any legal agreement, including an agreement that you or a subrecipient considers a contract.

4. Subrecipient means an entity that:

- i. Receives a subaward from you (the recipient) under this award; and
- ii. Is accountable to you for the use of the Federal funds provided by the subaward.

5. Total compensation means the cash and noncash dollar value earned by the executive during the recipient's or subrecipient's preceding fiscal year and includes the following (for more information see 17 CFR 229.402(c)(2)):

- i. Salary and bonus.
- ii. Awards of stock, stock options, and stock appreciation rights. Use the dollar amount recognized for financial statement reporting purposes with respect to the fiscal year in accordance with the Statement of Financial Accounting Standards No. 123 (Revised 2004) (FAS 123R), Shared Based Payments.
- iii. Earnings for services under non-equity incentive plans. This does not include group life, health, hospitalization or medical reimbursement plans that do not discriminate in favor of executives, and are available generally to all salaried employees.
- iv. Change in pension value. This is the change in present value of defined benefit and actuarial pension plans.
- v. Above-market earnings on deferred compensation which is not tax-qualified.
- vi. Other compensation, if the aggregate value of all such other compensation (e.g. severance, termination payments, value of life insurance paid on behalf of the employee, perquisites or property) for the executive exceeds \$10,000.

End of clause

9. FINANCIAL ASSISTANCE USE OF UNIVERSAL IDENTIFIER AND CENTRAL CONTRACTOR REGISTRATION (2 CFR Part 25)

Appendix A to Part 25--Award Term

I. Central Contractor Registration and Universal Identifier Requirements

A. Requirement for Central Contractor Registration (CCR). Unless you are exempted from this requirement under 2 CFR 25.110, you as the recipient must maintain the currency of your information in the CCR until you submit the final financial report required under this award or receive the final payment, whichever is later. This requires that you review and update the information at least annually after the initial registration, and more frequently if required by changes in your information or another award term.

B. Requirement for Data Universal Numbering System (DUNS) Numbers. If you are authorized to make subawards under this award, you:

1. Must notify potential subrecipients that no entity (see definition in paragraph C of this award term) may receive a subaward from you unless the entity has provided its DUNS number to you.
2. May not make a subaward to an entity unless the entity has provided its DUNS number to you.

C. Definitions. For purposes of this award term:

1. Central Contractor Registration (CCR) means the Federal repository into which an entity must provide information required for the conduct of business as a recipient. Additional information about registration procedures may be found at the CCR Internet site (currently at <http://www.ccr.gov>).

2. Data Universal Numbering System (DUNS) number means the nine-digit number established and assigned by Dun and Bradstreet, Inc. (D&B) to uniquely identify business entities. A DUNS number may be obtained from D&B by telephone (currently 866-705-5711) or the Internet (currently at <http://fedgov.dnb.com/webform>).

3. Entity, as it is used in this award term, means all of the following, as defined at 2 CFR part 25, subpart C:

- a. A Governmental organization, which is a State, local government, or Indian Tribe;
- b. A foreign public entity;
- c. A domestic or foreign nonprofit organization;
- d. A domestic or foreign for-profit organization; and
- e. A Federal agency, but only as a subrecipient under an award or subaward to a non-Federal entity.

4. Subaward:

a. This term means a legal instrument to provide support for the performance of any portion of the substantive project or program for which you received this award and that you as the recipient award to an eligible subrecipient.

b. The term does not include your procurement of property and services needed to carry out the project or program (for further explanation, see Sec. ----.210 of the attachment to OMB Circular A-133, "Audits of States, Local Governments, and Non-Profit Organizations").

c. A subaward may be provided through any legal agreement, including an agreement that you consider a contract.

5. Subrecipient means an entity that:

- a. Receives a subaward from you under this award; and

- b. Is accountable to you for the use of the Federal funds provided by the subaward.

End of Clause

10. AWARD CLOSE OUT

a. The following documents shall be submitted within 90 calendar days of the end of the term of the award:

(1) Final SF425, "Federal Financial Report" (form available on web site <https://www.usamraa.army.mil>)

(2) Final Technical Report

(3) Final DD Form 882, "Report of Inventions and Subcontracts" (form available on web site <https://www.usamraa.army.mil>)

(4) Cumulative listing of only the nonexpendable personal property acquired with award funds for which title has not been vested to the recipient, if applicable. (This may be submitted on institution letterhead.)

(5) "Volunteer Registry Data Sheet," USAMRDC Form 60-R, if applicable. (Form available on the USAMRMC ORP web site <https://www.usamraa.army.mil/pages/pdf/60r.pdf>.) The PI shall complete a form for each subject enrolled in this study and forward in accordance with ORP requirements.

b. In the event a final audit has not been performed prior to the closeout of the award, the sponsoring agency retains the right to recover an appropriate amount after fully considering the recommendations on disallowed costs resulting from the final audit.

c. The recipient shall promptly refund any unspent balances of funds the DOD Component has advanced or paid that is not authorized to be retained by the recipient. **Make check payable to the U.S. Treasury and mail to:**

USAMRAA
Attn: MCMR-AAA-S
Award No. W81XWH-11-1-0809
820 Chandler Street
Fort Detrick, Maryland 21702-5014

(End of Summary of Changes)

SUPPORTING DATA:

None other than those contained with the BODY section of this Annual Report.